Total RNA Isolation

1) Prepare PBMCs samples for isolation of RNA.
2) Lyse the cells in 1 ml of Trizol reagent and incubate the homogenates for 5 minutes at room temperature to permit complete dissociation of nucleoprotein complexes.
3) Mix the samples and transfer them to a 1.5ml eppendorf tube.
4) Add 0.2 ml of chloroform per milliliter of samples. Mix the samples by vigorous shaking or vortexing for 30 seconds and then stand for 3 minutes.
5) Separate the mixture into two phases by centrifuging at 12,000 rpm for 15 minutes at 4°C. Transfer the upper aqueous phase to a fresh 1.5ml eppendorf tube.
6) Precipitate the RNA from the aqueous phase: add equal volume of isopropanol with each upper aqueous phase of procedure 5. After thorough mixing, store the final solution for 10 minutes at room temperature.
7) Separate the mixture into two phases by centrifuging at 12,000 rpm for 15 minutes at 4°C. Remove the upper aqueous phase.
8) Wash the pellet twice with 1ml 75% ethanol. Collect the precipitated RNA by centrifugation at 8,500 rpm for 5 minutes at 4°C in a microfuge.
9) Wash the pellet twice with 75% ethanol, and centrifuge again.
10) Remove ethanol with a disposable pipette tip.
11) Let the ethanol evaporate. Do not allow pellet to dry completely.
12) Add 10-15 µl of DEPC-treated H_2O. Solute the RNA at 4°C for 10 minutes.
13) Estimate the concentration of the RNA by measuring the absorbance at 260 nm of an aliquot of the final preparation.